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Seasonal Variation in Conduction Velocity of Action Potentials in Squid Giant Axons

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Abstract. To determine whether the electrical properties of the squid giant axon are seasonally acclimated, action potentials, recorded at different temperatures, were compared between giant axons isolated from *Loligo pealei* caught in May, from relatively cold waters (~10°–12°C), and in August, from relatively warm waters (~20°C). Parameters relating to the duration of the action potential (*e.g.*, maximum rate of rise, maximum rate of fall, and duration at half-peak) did not change seasonally. The relationship between conduction velocity and temperature remained constant between seasons as well, in spite of the fact that May axons were significantly larger than August axons. When normalized to the fiber diameter, mean May conduction velocities were 83% of the August values at all temperatures tested, and analysis of the rise time of the action potential foot suggested that a change in the axoplasmic resistivity was responsible for this difference. Direct measurements of axoplasmic resistance further supported this hypothesis. Thus seasonal changes in the giant axon's size and resistivity are not consistent with compensatory thermal acclimation, but instead serve to maintain a constant relationship between conduction velocity and temperature.

Introduction

Signal transduction in the nervous system is profoundly temperature sensitive. Acclimation of higher order nervous function to variation in environmental temperature has been the subject of many investigations (see Prosser and Nelson, 1981); however, the precise mechanisms of such acclimations are not well understood. Do they involve changes at the level of the action potential, the synapse, or both? Action potential duration and propagation are strongly influenced by acute temperature changes (Hodgkin and Katz, 1949), largely due to temperature sensitivity of the underlying ion channels (Hodgkin *et al.*, 1952). If action potentials themselves are a common target for thermal acclimation, which properties are affected? In the giant nerve fibers of earthworms, cold acclimation speeds the action potential duration, conduction velocity, and refractory period *vs.* temperature relationship, but not to the extent that, when measured at rearing temperatures, the kinetics of cold- and warm-acclimated worms are equivalent (Lagerspetz and Talo, 1967; Talo and Lagerspetz, 1967). In goldfish cardiac muscle cells, the action potential's duration is reduced in cold-acclimated fish (Ganim *et al.*, 1998). In certain *Aplysia* neurons, however, early potassium current kinetics are not affected by rearing temperature (Treistman and Grant, 1993).

The squid giant axon, long a model for understanding the basic physiology of voltage-dependent ion channels, is, for a variety of reasons, an excellent system for examining temperature-dependent acclimation of the action potential. First, its output participates in a known function—the jet-propelled escape response (Prosser and Young, 1937; Young, 1938; Otis and Gilly, 1990)—and presumably it is advantageous for this response to be rapid. Second, the dimensions of the giant axon permit action potentials, macroscopic ionic currents, gating currents, and single-channel currents to be measured from the same preparation (Hodgkin and Huxley, 1952; Armstrong and Bezanilla, 1973; Conti and Neher, 1980; Bezanilla, 1987). Third, Na and K currents, which underlie the action potential, have been extensively characterized in this system (see Gilbert *et al.*, 1990). Fourth, giant axon Na and K channels have been

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Abbreviations: C_m, membrane capacitance; MRF, maximum rate of fall; MRR, maximum rate of rise; R_e, external resistivity; R_i, internal resistivity; r_e, external resistance; r_i, internal resistance.

defined on a molecular level (Rosenthal and Gilly, 1993; Rosenthal *et al.*, 1996). Finally, and perhaps most important, squid of the genus *Loligo* (and other genera that contain "giant" axons) inhabit a wide variety of thermal environments.

Squid of the species *Loligo pealei* live off the eastern seaboard of North America, where inshore water temperatures undergo large seasonal fluctuations. By examining the axons from these squid in both May and August, the present study seeks to identify those properties of the action potential that change on a seasonal basis.

Materials and Methods

Squid collection and water temperatures

Adult specimens of *Loligo pealei* were collected from the waters surrounding Woods Hole, Massachusetts, in 1997 and 1998 during May and August. In May, specimens were jigged from the town dock, and in August they were caught by trawls in Vineyard Sound. During two trawls, water temperature was measured at the net opening with a data logger. Daily temperatures, recorded near the Marine Biological Laboratory (MBL) seawater intake system at a depth of 15 feet, were kindly furnished by Janice Hanley, MBL water quality technician. Data used in Figure 2 were recorded by the National Oceanic and Atmospheric Administration (NOAA) at the Woods Hole Oceanographic Institution pier (tide station number 8447930, available on the NOAA website). Squid were maintained in flowing seawater, whose temperature was kept within 1 degree of the intake temperature, and were used within 2 days of capture. Animals were killed by rapid decapitation, and hindmost stellar nerves were removed and carefully cleaned of small fibers in seawater. All experiments, unless otherwise specified, were performed in 10 K^+ artificial seawater (ASW; composition in millimoles: 430 NaCl, 10 KCl, 50 $MgCl_2$, 10 $CaCl_2$, 10 HEPES, pH 7.5, 970 mOsm).

Action potential measurements

Propagated action potentials were measured by mounting a freshly dissected axon (4–6 cm) in a long, rectangular glass chamber filled with 10 K^+ ASW. Temperature was regulated using two Peltier units mounted under the chamber and was measured with a hand-held thermocouple positioned directly adjacent to the axon. After equilibration of the chamber, temperatures at all points along the axon were within ±0.5°C of the recorded temperature. Action potentials were stimulated intracellularly at one end of the axon using brief current pulses (2–20 μA for 200–400 μs) administered through a 0.4–0.6 MΩ micropipette filled with 3 *M* KCl, and connected to the output of the online D/A converter. Stimuli, which varied from axon to axon, were the minimum required to produce a consistent action potential. Voltage signals were recorded at two points along the axon using two additional micropipettes (3 *M* KCl, 1–3 MΩ) connected to two high-impedance, capacitance-compensated electrometers. The chamber was grounded using two Ag^+/AgCl coils embedded in 10 K^+ ASW + 3% agarose and connected to virtual ground. Signals were collected using software written in-house and a PC44 (Innovative Technologies) signal processor board connected to a PC compatible computer. Sampling rates varied between 200 kHz and 1 MHz, depending on the temperature, and signals were filtered at 1/10 of the sampling rate. Axon diameters and distances between micropipettes were measured with an eyepiece micrometer. Data were analyzed only from electrode impalements with resting potentials more hyperpolarized than −55 mV.

Capacitance and resistance measurements

Membrane capacitance was measured in the voltage-clamp configuration as previously described (Bezanilla *et al.*, 1982a, b) with minor modifications. Signals were collected as described in the previous section. A 75-μm platinum wire for passing current and an internal measuring electrode consisting of an 80-μm glass capillary filled with 0.6 *M* KCl and containing a floating 25-μm platinum wire were inserted into the axon in a piggy-back configuration (Hodgkin *et al.*, 1952). A wide-aperture glass capillary filled with 10 K^+ ASW + 3% agar was used as an external reference. Capacity transients were generated by brief pulses from a holding potential of −80 mV to −90 mV. The average of 10 such records was used for analysis. Data were collected at 500 kHz and filtered at 50 kHz. For resistance measurements, axons were carefully cleaned of all adhering accessory fibers and placed on a platform of clear acrylic plastic. The axon ends were then cut and dipped into two pools containing 500 m*M* K-glutamate, 10 HEPES, 5 EGTA, pH 7.5. These pools were voltage clamped, and signals were collected, as described above. In all cases, data were analyzed using in-house software. Error bars represent the standard error of the mean (SEM), and a Student's *t* test was used where probabilities are indicated in the text.

Results

In the giant axon, action potential duration and conduction velocity are strongly affected by temperature (Hodgkin and Katz, 1949; Chapman, 1967). Figure 1A shows three action potentials, recorded at different temperatures, from a single electrode inserted into a giant axon. Clearly, the durations of the rising and falling phase both increase as the temperature is decreased. The half-width of the action potential recorded at 20°C is 365 μs. This number increases to 850 μs at 10.2°C, and to 2400 μs at 1.8°C. Furthermore, the conduction velocity shows a similar temperature dependence. In Figure 1B, action potentials, evoked by a single

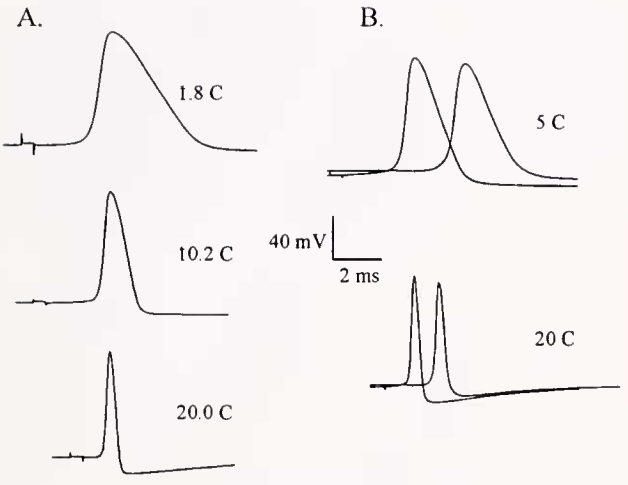

Figure 1. Action potentials recorded at different temperatures. (A) Propagated action potentials from a single position in a single giant axon measured at three temperatures. (B) Propagated action potentials recorded at two positions in a single axon measured at two temperatures. Axons were bathed in 10 K^+ artificial seawater. Brief transients at the beginning of records are stimulus artifacts.

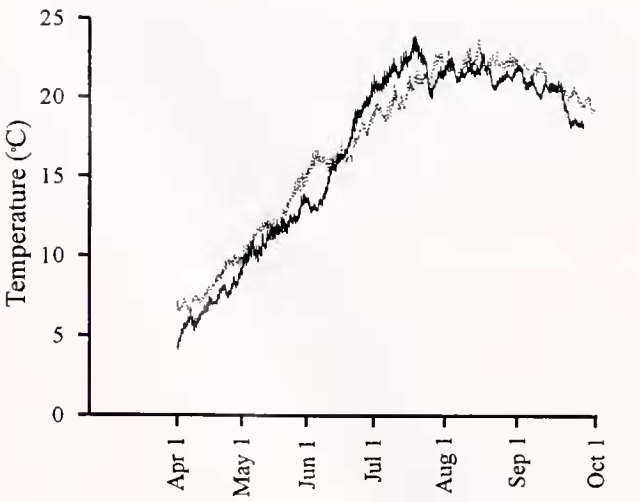

Figure 2. Seasonal temperature variation in the Woods Hole Passage. Hourly temperature records from April through September for 1997 (solid line) and 1998 (dotted line). Temperatures were taken from NOAA station number 8447930 located at the Woods Hole Oceanographic Institution at 41° 31.5' N, 70° 40.3' W.

stimulus, are recorded at two points, separated by 19.6 mm, along the same axon. From this experiment the conduction velocity is calculated to be 9.56 m/s at 5°C and 18.94 m/s at 20°C. The axons used in this figure were dissected from a squid captured in May.

Water temperatures in the Woods Hole Passage and in Vineyard Sound, where squid for these studies were captured, change dramatically between the spring and summer. In May, squid were collected by jigging from waters directly measured to be 10°–12°C. In late August, squid were captured by trawling, and on two trawls the average temperature at the net opening was recorded to be 20.4°C and 21.2°C. Figure 2 shows hourly water temperatures recorded by the NOAA tidal monitoring station (adjacent to the Woods Hole Oceanographic Institution pier in the Woods Hole Passage) between April and October, 1997 and 1998. During May, water temperatures vary between about 10° and 12°C. In August they average slightly greater than 20°C. These values are corroborated by daily temperature records taken near the MBL seawater intake system at a depth of 15 feet. In 1998, May and August average temperatures (±SD) were 12.4°C ± 1.2 and 21.8°C ± 0.49, respectively. The few data points available for this site in 1997 are similar to the 1998 values (10.2°C on May 5, 12.8°C on May 28, 22.0°C on August 8, and 21.1°C on August 26). Thus, water temperatures during the study period in both 1997 and 1998 changed by about 10°C. Acute temperature changes of this magnitude would clearly affect the giant axon's electrical properties (see Fig. 1). Do these properties change to compensate for seasonal temperature variation?

To answer this question, action potentials were compared between squid captured in May and in late August. Figure 3 compares maximal rates of rise (MRR) and fall (MRF) of the action potential, at various temperatures, for May and August squid. No significant difference exists for either measurement between groups. MRRs have a nearly linear

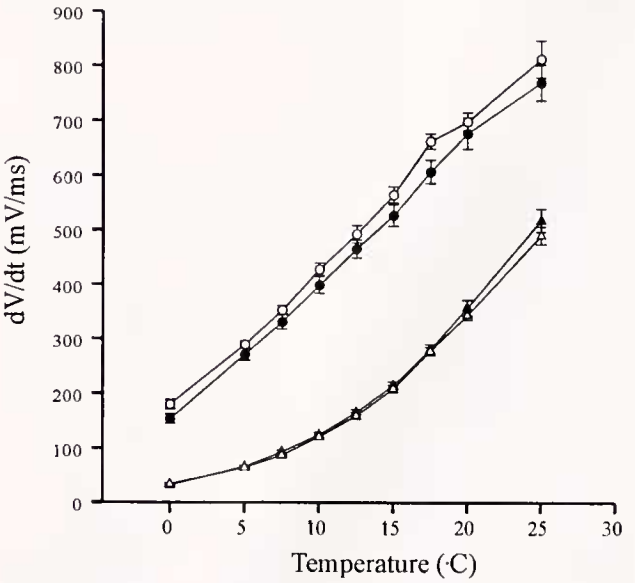

Figure 3. Action potential maximum rates of rise and fall do not change seasonally. Action potential records from squid in May (filled symbols) and August (open symbols) were differentiated numerically, and maximum values (rates of rise; circles) and minimum values (rates of fall, absolute values; triangles) were plotted against temperature. Action potentials were recorded at two positions along an axon. Error bars represent the standard error of the mean; n = 10 records (5 axons) for May and 11 records (6 axons) for August.

temperature relationship, while that of the MRFs is exponential. In addition, the MRFs have a higher temperature coefficient. Q_{10} values for the MRF are 3.7 and 2.5 for the temperature ranges 0°–12.5°C and 12.5°–25°C, respectively. For the same intervals, Q_{10}'s for the MRR are 2.4 and 1.5. Other measurements relating to the action potential duration (*e.g.*, rise time, fall time, and duration at half-peak amplitude) also showed no seasonal difference.

In contrast, resting potentials and conduction velocities did exhibit significant seasonal variation. Figure 4A compares the resting potential *vs.* temperature relationship for May and August axons. Between 0° and 15°C, both groups

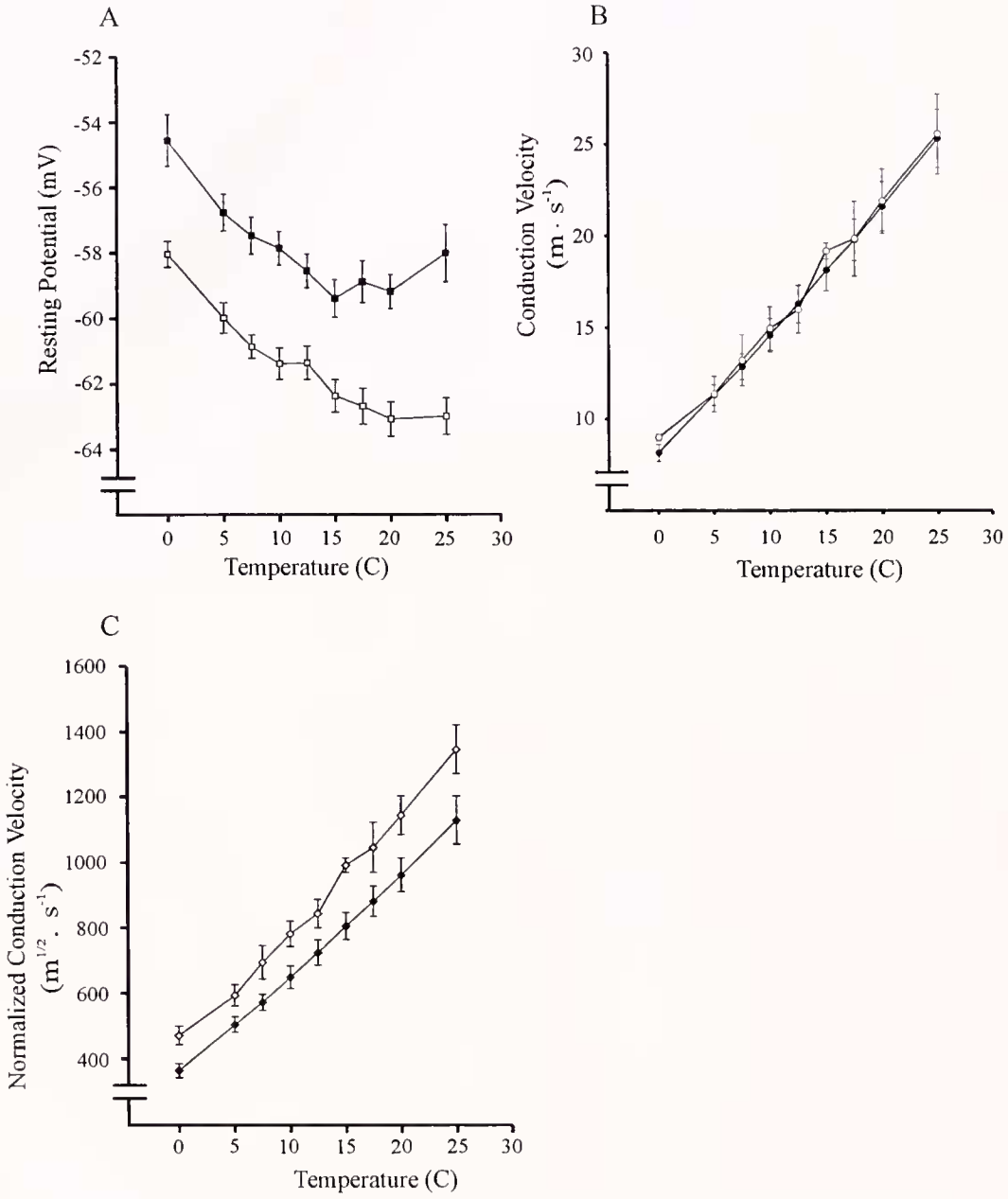

Figure 4. Resting potentials and conduction velocities change seasonally. (A) Resting potential *vs.* temperature relationship for May axons (filled symbols) and August axons (open symbols). Error bars represent the standard error of the mean (SEM); $n = 10$ for each season. (B) Conduction velocities *vs.* temperature for May and August axons (same symbol convention) as in A. (C) Conduction velocities, normalized to the square root of the diameter, *vs.* temperature for May and August axons (same symbol convention). Error bars represent SEM; $n = 6$ for each season for both B and C.

become more hyperpolarized as the temperature is raised. In this temperature range, the May axons are on average ~3 mV more depolarized. At temperatures greater than 15°C, May axons become progressively more depolarized, while August axons continue to hyperpolarize until approximately 20°C, after which they level out. Thus at 25°C the resting potential disparity reaches ~5 mV.

Figures 4B and 4C show the relationship between conduction velocity and temperature. Absolute (non-normalized) conduction velocities were equivalent between May and August squid (4B) at all temperatures tested. Interestingly, May axons had significantly larger diameters (506 ± 26.8 μm; mean ± SEM) than those in August (383 ± 14.68 μm; mean ± SEM). Conduction velocity in the giant axon is proportional to the square root of the axon diameter, a relationship originally established by Hodgkin and Huxley (1952) and verified by many other groups (Chapman, 1967; Taylor, 1963). Taking this into account, normalized conduction velocities are plotted *vs.* temperature in Figure 4C. Conduction velocities from May axons are relatively slow, being on average 83% ± 2.5% (SD) of the August values. In both seasons the Q_{10} values were equivalent (1.5 between 10° and 20°C).

The fact that normalized conduction velocity values changed from May to August, but the MRR and MRF did not, suggested that the passive electrical properties of the axon had also changed. The action potential's initial rate of rise, or "foot," can be used to extract information related to the axon's cable properties by the following relationship:

$$C_m(r_i + r_e) = 1/\tau\theta^2$$

where C_m is membrane capacitance, r_i is internal resistance, r_e is external resistance, τ is the time constant of the rise time of the action potential foot, and θ is the conduction velocity (Taylor, 1963). Figure 5A shows an example of an exponential fit to the foot of an action potential recorded at 10°C from a May axon. In this case, τ was 210 μs, θ was 12.45 m/s, and therefore $C_m(r_i + r_e)$ was 30.7 Ω*F*cm. Similar analysis was extended to action potentials from May and August, and all data were normalized to account for axons of different diameters (Fig. 5B). At all temperatures, normalized $C_m(r_i + r_e)$ was greater in May than in August axons. On average, May values were 31.7% ± 6% (SD) greater.

The preceding analysis indicated that the product of resistance and capacitance was variable between seasons; however, it did not identify which property changed. To accomplish this, capacitance and resistance were measured independently. Capacitance was measured at a variety of temperatures using a conventional axial wire voltage clamp. A Q_{10} of 1.06 was determined for the relationship between capacitance and temperature in two axons (data not shown). This number agrees well with previously published data from squid (Taylor *et al.*, 1962) and was used to extrapolate all experimental values to 15°C. For May and August axons, mean capacitance was determined to be 1.03 ± 0.039 μF/cm^2 (SEM, n = 6) and 0.96 ± 0.027 μF/cm^2 (SEM, n = 6), respectively. A statistical difference between these means is not well supported (P = 0.18). Thus differences in capacitance are not sufficient to account for the $C_m(R_i + R_e)$ data from the previous section.

Axoplasmic resistance was measured directly in dissected axons. Axons were blotted dry and placed on an acrylic plastic platform; their ends were cut and dipped into two reservoirs containing internal solution (Fig. 6A). The reservoirs were then voltage clamped, and after transients had subsided, the current flow through the axon was measured and normalized to the axon's cross sectional area and length. All measurements were conducted at room temperature (about 20°C). The results from a typical axon segment of 404 μm diameter and 3.65 cm length are shown in Figure 6B. In this case, the voltage between the reservoirs and the current flow through the axon were 107.3 mV and 1.24 μA. Thus the resistance ($r_i + r_e$) for this axon was calculated to be 86.5 kΩ, and the specific resistance ($R_i + R_e$) was 30.3 Ω*cm. As expected, the current voltage relationship at a variety of test potentials is linear (Fig 6C). A series of axons from May and August were analyzed in a similar manner and their specific resistivities were found to differ. Mean $R_i + R_e$ was measured to be 35.2 ± 1.3 Ω*cm (± SEM, n = 6) and 28.4 ± 2.5 (± SEM, n = 6; P = 0.05) in May and August, respectively. Thus on average, May values are 22% greater than August values.

Discussion

The present investigations were initiated to identify seasonal changes in the giant axon's electrical properties, and to address whether these changes could compensate for seasonal temperature variability. Implicit in these studies is that the squid do in fact experience seasonal temperature variability. Temperature data from all sources, taken at various depths, all show a temperature profile similar to that in Figure 2 (*i.e.*, an approximate 10°C difference between May and August). The recorded spring temperatures are probably maximum values and thus are a conservative estimate of the squid's environment. Summer temperatures reported in this paper are probably representative for Vineyard Sound and the Woods Hole Passage, the locations where the squid used for these experiments were captured. Turbulence, created by large tidal flows, prevents the formation of thermoclines in these shallow areas, and temperatures are uniform throughout the water column. Other papers have come to a similar conclusion about mixing and report summer temperatures as high as 23°C (Summers, 1968; McMahon and Summers, 1971). Outside of the Vineyard Sound, temperatures are likely to be significantly

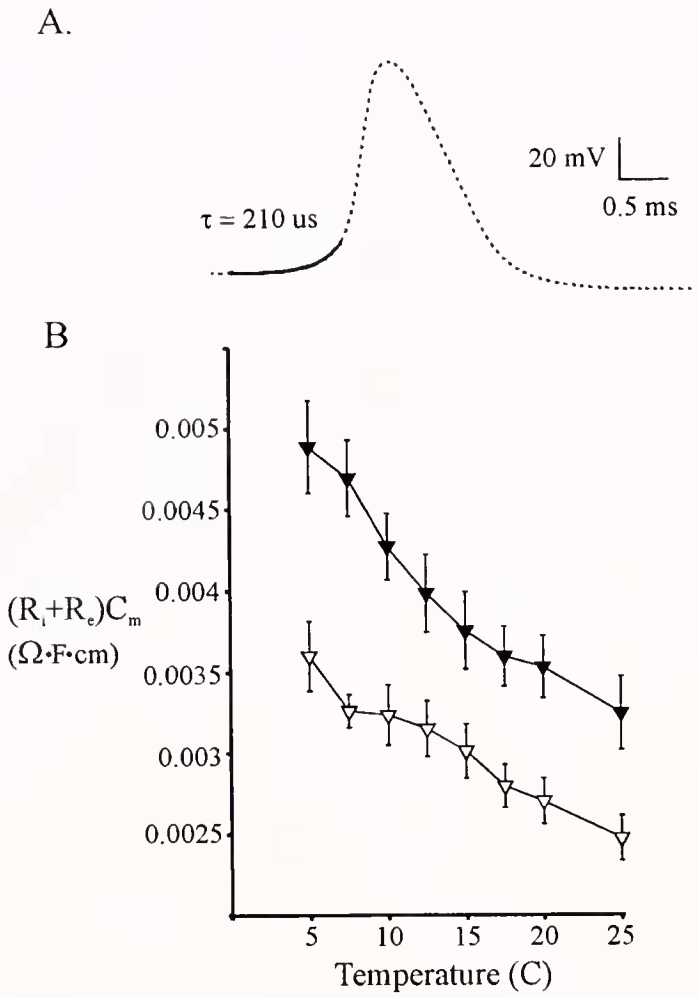

Figure 5. Product of resistance and capacitance changes seasonally. (A) Example of measurement. Dotted line is an action potential recorded at 10°C from a May axon; solid line is an exponential fit to the action potential foot. (B) Values of $(R_i + R_e)C_m$ *vs.* temperature for May (filled triangles) and August (open triangles) axons. See text for derivation of $(R_i + R_e)C_m$. Error bars represent the standard error of the mean; $n = 6$ for May and 5 for August.

lower. Schopf (1967) reports the presence of thermoclines and maximum annual bottom-water temperatures of 13°C in the waters off Nantucket.

The migration patterns of *Loligo pealei* are not well understood. On a seasonal basis this species is reported to winter near the break of the continental shelf where it can avoid temperatures below 8°C (Summers, 1969). In the spring these squid move inshore when waters warm past about 10°C (Summers, 1969; Mesnil, 1977). The first group to arrive are the 2-year-olds, normally in early May, followed by the 1-year-olds in June (Summers, 1971). This sequence of events probably explains the larger diameter of the May axons. Migration on a shorter time scale has not been reported for this species, and therefore it is unknown whether these squid migrate to colder oceanic waters on a daily basis. We consider such a migration unlikely for two reasons. First a substantial horizontal shift would be required to reach deep oceanic waters (depending on the point of departure in Vineyard Sound). Second, the August squid were routinely captured during the day. In other cephalopods, daily migrations involve a nocturnal shift to shallow waters (Boyle, 1983; Hanlon and Messenger, 1996). Therefore it is likely that the squid spend a significant portion of their time at the water temperatures specified in this report. Various reports document the presence of *L. pealei* in yet warmer waters (*e.g.*, 22°–29°C in the Gulf of Mexico; see Boyle, 1983).

This study presents no evidence for a seasonal compensation in the propagated action potential's duration, as there is no change in the curve of MRR or MRF *vs.* temperature. These data also suggest that the properties of the underlying ionic current do not change, and studies using voltage-clamped giant axons support this conjecture (data not shown). Therefore it is predicted that *in vivo*, the duration of

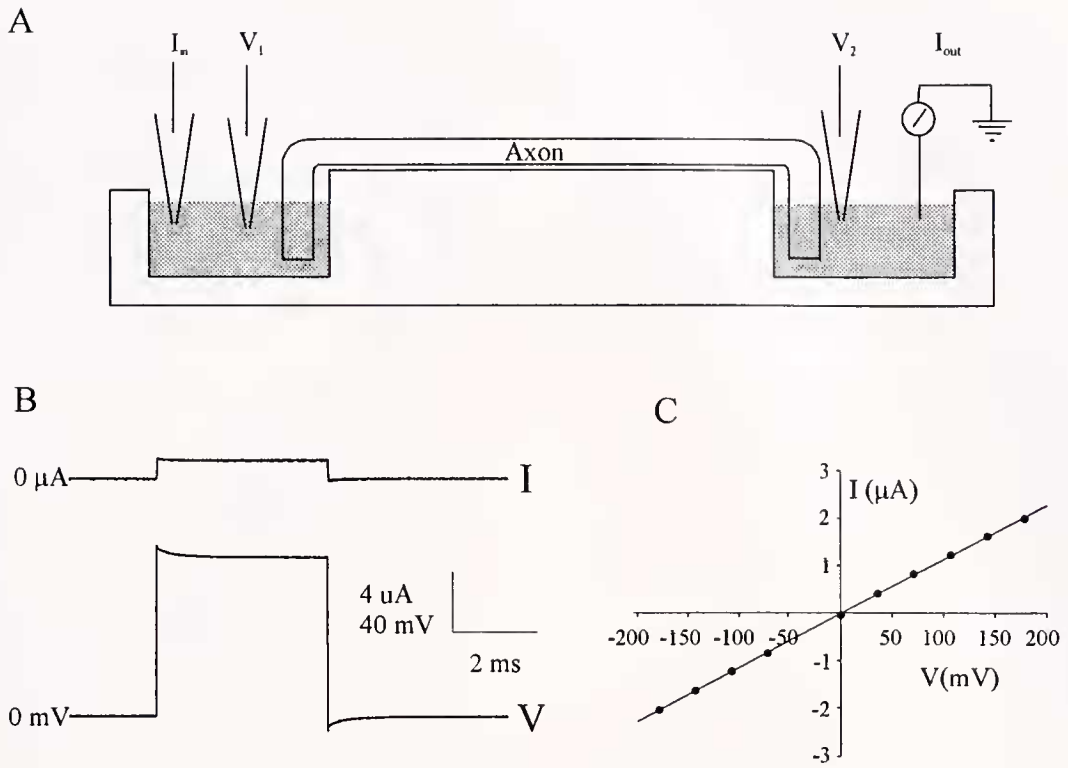

Figure 6. Direct resistance measurements. (A) Schematic of experimental setup. A defined length of axon was placed on a clear plastic platform, and each end was cut and placed in a bath containing internal solution (in m*M*: 500 K-glutamate, 10 HEPES, 2.5 EGTA, pH 7.5). The interbath voltage was clamped using a home-built squid axon apparatus, and the resulting current was measured. (B) An example of the current resulting from a 100-mV voltage step (May axon). (C) Current-voltage relationship from the same axon. All experiments performed at temperatures between 18° and 20°C.

the action potential in May squid is over twice as long as it is in August squid (see Fig. 1 for examples of action potentials recorded at 10°C and 20°C). Unlike the action potential duration, the conduction velocity does appear to be regulated between seasons. However, the direction of the change is not consistent with a compensatory thermal acclimation: despite a seasonal disparity in axon diameter, the relationship between conduction velocity and temperature remains constant, due mostly to resistive changes in the axon. Computer simulations of conduction velocities using the Hodgkin and Huxley equations (Hodgkin and Huxley, 1952) support this assertion. By substituting the May and August values for $C_m(R_i + R_e)$, determined by fits to the action potential foots (32.4 ΩF and 42.7 ΩF, respectively, at 10°C), there is a 14.2% increase in conduction velocity. The difference determined from direct measurements of conduction velocities in May and August axons at 10°C was 14.6%.

Fits to the action potential foot predicted that the product of $(R_i + R_e)$ and C_m increased by approximately 30% between August and May. This is in reasonable agreement with direct measurements of $(R_i + R_e)$ for the same seasons, which increased by 22%. Capacitive changes were not found to be statistically significant. It is probable that resistive changes are due to changes in R_i, as the contribution of R_e to our measurements is expected to be very small. Great care was taken to blot the axon's external surface prior to recording, thus the layer of adhering seawater would be quite small compared to the cross-sectional area of the axon. Cole and Hodgkin, who employed a similar experimental setup, came to the same conclusion (Cole and Hodgkin, 1939). In addition, our reported values of $R_i + R_e$, particularly those for August (28.7 Ω•cm), are consistent with previously reported values of R_i. Cole and Hodgkin reported 29 Ω•cm (Cole and Hodgkin, 1939) and in a separate report, Cole reported the resistivity of extruded axoplasm to be 28 Ω•cm (Cole, 1975). Using two internal microelectrodes, Carpenter *et al.* (1975) reported it to be 31 Ω•cm. It is unclear during which season these studies were conducted.

Seawater, which is isosmotic with axoplasm (Gilbert *et al.*, 1990), has a specific resistivity of only 20 Ω•cm (Cole and Hodgkin, 1939). Why is axoplasm a relatively poor conductor? First, unlike seawater, axoplasm contains mostly large organic anions that have lower mobilities than chloride. In addition, our measurements of

resistivity consider the axon as a conducting cylinder, and do not take into account the organelles, which occupy an unknown percentage of the volume. Finally, axoplasm contains a good deal of immobile protein. We observed that axoplasm appears gelatinous in May, whereas in August it is considerably more liquid, possibly due to a decrease in the order of the underlying cytoskeletal proteins. Differences in any of these parameters could underlie the seasonal differences in axoplasmic resistivity.

In the absence of a seasonal acclimation, giant axon action potentials would travel much faster in August than in May. The resistive changes discussed in this paper would not help compensate for temperature changes in conduction velocity and therefore they do not contribute to a compensatory acclimation. However, the giant axon is clearly a part of a larger motor system, the pieces of which do not have equal temperature sensitivities. Perhaps thermal acclimation involves maintaining specific ratios between the rates of processes. For example, the temperature dependence of the giant synapse is steep compared to the various properties related to the action potential discussed in this paper (see Llinas, 1999). Between 10° and 20°C, synaptic delay at the giant synapse has a Q_{10} of 3.8 (Llinas *et al.*, 1987) as compared with the Q_{10} of 1.5 measured for action potential conduction velocity in this work. Therefore the ratio of synaptic transmission to conduction velocity, which may be important for integration in the nervous system, would be greater in August than in May. The resistive changes discussed in this paper would help to maintain a more similar ratio between seasons. In support of this conjecture it is noteworthy that the non-normalized relationship between conduction velocity and temperature did not vary between May and August, in spite of the fact that August axons were significantly smaller.

Acknowledgments

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